Treatment of Metastatic Melanoma with Autologous CD4+ T Cells against NY-ESO-1

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SUMMARY

We developed an in vitro method for isolating and expanding autologous CD4+ T-cell clones with specificity for the melanoma-associated antigen NY-ESO-1. We infused these cells into a patient with refractory metastatic melanoma who had not undergone any previous conditioning or cytokine treatment. We show that the transferred CD4+ T cells mediated a durable clinical remission and led to endogenous responses against melanoma antigens other than NY-ESO-1.

CD8+ cytotoxic T cells can be harvested from a patient with cancer, expanded in vitro, selected for specificity against a tumor-associated antigen, and infused back into the patient. Such autologous T cells have been shown in clinical trials to have a beneficial effect in some patients with cancer.[1–5] The cytotoxic antitumor effect of CD8+ T cells depends on CD4+ T cells, which provide CD8+ T cells with growth factors such as interleukin-2 and can mediate the destruction of tumor cells either directly or indirectly.[5–8] Growth factors such as interleukin-2 can act in an autocrine manner, which in principle would allow an infusion of CD4+ T cells to proliferate in the patient and stimulate endogenous antitumor CD8+ T cells. Until recently, however, a means of isolating and expanding antitumor CD4+ T cells in numbers sufficient for cellular therapy has not been feasible. The identification of HLA class II–restricted epitopes in tumor-associated antigens such as NY-ESO-1 and tyrosinase and the development of methods for the isolation and expansion of CD4+ T cells in vitro gave us the opportunity to implement a clinical trial to evaluate the safety, in vivo persistence, and antitumor efficacy of autologous CD4+ T-cell clones for the treatment of metastatic melanoma. We describe a patient with refractory metastatic melanoma who had a long-term complete remission after receiving autologous NY-ESO-1–specific CD4+ T-cell clones.

CASE REPORT

The patient was 52 years old when he presented with recurrent melanoma and pulmonary and left iliac and inguinal metastases. His tumor had not responded to high-dose interferon alfa, four cycles of high-dose interleukin-2, and local excision. Baseline staging with magnetic resonance imaging of the brain and computed tomography (CT) of the chest, abdomen, and pelvis showed metastatic disease in the posterior right pleura, right hilum, and

left iliac region. There were no central nervous system metastases. Positron-emission tomography (PET) of the entire body revealed no other sites of uptake.

Immunohistochemical analysis that was performed on a biopsy specimen of the inguinal tumor revealed uniform expression of melanoma antigen recognized by T cells (MART-1, also called melanoma tumor antigen [MelanA]), 3+ staining of melanoma antigen 3 (MAGE-3), 3+ staining of NY-ESO-1, and no detectable glycoprotein 100. The patient was enrolled in a clinical trial of autologous T-cell therapy for melanoma at the Fred Hutchinson Cancer Research Center in Seattle (protocol 1585). The study was approved by the institutional review board, and the patient provided written informed consent.

Peripheral-blood mononuclear cells were collected by leukopheresis, and T cells in the harvest were cultured. Since the patient's HLA genotype included the HLA-DOB1*0401 allele, CD4+ T-cell clones targeting the DPB1*0401-restricted epitope of a peptide derived from NY-ESO-1 were isolated from the culture and expanded for cellular therapy. In vitro, these clones responded to the NY-ESO-1 peptide by producing interleukin-2 and interferon-γ. During a 2-hour period, the patient received a single infusion of 3.3×10^9 clonal CD4+ T cells per square meter of body-surface area, totaling 5 billion T cells. No preinfusion conditioning or postinfusion interleukin-2 was administered.

Transient lymphopenia, low-grade fever (maximum temperature, <38.5°C), and myalgia, all consistent with cytokine release,[3] developed and resolved within 3 days after the infusion. There were no serious adverse events, according to the Common Toxicity Criteria of the National Cancer Institute. Two months after the infusion, restaging PET and CT scans revealed complete resolution of pulmonary and nodal disease; there was no evidence of abnormal fluorodeoxyglucose uptake and no other radiographic or clinical evidence of disease (Fig. 1). The patient remained disease-free 2 years later.

METHODS

GENERATION OF ANTIGEN-SPECIFIC CD4+ T-CELL CLONES

Antigen-specific CD4+ T-cell clones were generated in a manner similar to that for CD8+ T cells described previously.[3,9] Briefly, autologous monocyte-derived dendritic cells were pulsed with 40 µg of the NY-ESO-1 peptide SLLMWITQCFLPVF[10] per milliliter and cocultured with the patient's T cells at a ratio of 10:1 (T cells:dendritic cells). Interleukin-2 (12.5 IU per milliliter) and interleukin-7 (10 ng per milliliter) were added every 3 days to maintain the growth of activated antigen-specific T cells. One week later, the T cells were restimulated with peptide-pulsed autologous mononuclear cells. One week after the second stimulation, T cells with specific reactivity against the NY-ESO-1 peptide were harvested and cloned at limiting dilution in the presence of irradiated feeder cells (mononuclear cells plus Epstein–Barr virus–transformed B cells), a monoclonal anti-CD3 antibody, and interleukin-2. Clones that showed a specific proliferative response to the NY-ESO-1 peptide in a thymidine incorporation assay were expanded in vitro. A total of four NY-ESO-1–specific CD4+ T-cell clones were generated from the patient. The clone that was selected for infusion could be expanded by a factor of 3000 to 5000 during a period of 18 days with the use of established methods.[11] Reactivity against NY-ESO-1 was confirmed after expansion by performing assays to detect antigen-specific proliferation and the release of interferon-γ (for details, see the Supplementary Appendix, available with the full text of this article at www.nejm.org).

DETECTION OF ANTIBODIES AGAINST TUMOR-ASSOCIATED ANTIGENS

An enzyme-linked immunosorbent assay was used to detect IgG antibodies against recombinant full-length NY-ESO-1, MAGE-3, and MART-1 proteins (1 µg per milliliter),

as described previously.[12] Reciprocal titers were extrapolated from serial dilutions, and titers of more than 100 were considered to be reactive. Specificity was determined by comparing reactivity with that of control antigens.

ASSAY FOR ANTIGEN-SPECIFIC RESPONSES

To evaluate the postinfusion T-cell responses to a panel of melanoma antigens, RNA-transfected autologous dendritic cells were used as stimulator cells in enzyme-linked immunospot (ELISPOT) assays. Melanoma antigens MART-1, glycoprotein 100, NY-ESO-1, and MAGE-3 were subcloned into the pGEM4Z vector, flanked by the signal sequence from lysosomal-associated membrane protein 1 (LAMP-1) to facilitate antigen sorting to both class I and class II antigen-presentation pathways (for details, see the Supplementary Appendix).[13] For the inteferon-γ ELISPOT assay, the patient's mononuclear cells were cocultivated with RNA-transfected dendritic cells at a ratio of 2:1 in triplicate filter wells. After overnight incubation, the plates were treated with a biotinylated anti–interferon-γ-detection antibody, clone 7B61 (Mabtech). Streptavidin HRP (Pharmingen) was added and developed to produce a color reaction with the use of the Vectastain AEC substrate kit (Vector Laboratories). Each spot on the filter well represented a single antigen-stimulated T cell. Spots were enumerated and the antigen-specific T-cell frequency was calculated as a fraction of the input number of mononuclear cells (see the Supplementary Appendix for details).

T-CELL TRACKING BY QUANTITATIVE PCR

We detected cells of the NY-ESO-1–specific CD4+ T-cell clone in the patient's blood using primers flanking the CDR3 region of the T-cell receptor of the clone. Genomic DNA from mononuclear cells was harvested and used as a template for a quantitative real-time polymerase-chain-reaction (PCR) assay. The frequency of the cells was determined by reconciling the melting temperature of the sample's reaction with that obtained from standards titrated with known frequencies of the CD4+ T-cell clone. This assay can detect a T-cell clone in peripheral blood at a sensitivity of 1:100,000 cells.

RESULTS

PERSISTENCE OF THE CD4+ T-CELL CLONE

The persistence of the infused NY-ESO-1–specific CD4+ T-cell clone in vivo was evaluated with the use of a quantitative PCR assay that can detect the clone-specific CDR3 region in samples of mononuclear cells. The T-cell clone was undetectable in preinfusion samples from the patient. After infusion, however, there was a rapid rise in the frequency of cells of the clone: they constituted almost 2.0% of all peripheral-blood mononuclear cells on day 3 and remained detectable in the patient's blood for more than 80 days (Fig. 2). During this time, the frequency of cells of the clone fluctuated between 0.7 and 3.0% of the total number of mononuclear cells.

CLINICAL EVALUATION

CT and PET scans obtained 2 months after the T-cell infusion revealed no evidence of disease, and follow-up after 22 months showed no evidence of recurrence (Fig. 1). At the time of the last contact with the patient, 26 months after the T-cell infusion, he had received no other treatment and had normal function, with a Karnofsky performance status of 90 to 100%. There were no detectable acute or long-term autoimmune-related toxic effects (e.g., dermatitis, vitiligo, uveitis, and orchitis).

T-CELL RESPONSES TO UNRELATED ANTIGENS

Even though only 50 to 75% of the tumor cells expressed NY-ESO-1, the entire tumor regressed after infusion of the NY-ESO-1–specific CD4+ T-cell clone. This inconsistency led us to postulate that the infused clone induced an immune response that was broader than expected. To test this hypothesis, we evaluated T-cell responses to two other melanoma-associated antigens that were expressed by the patient's tumor, MART-1 and MAGE-3. Using autologous dendritic cells that were engineered by RNA transfection to express these antigens, we could detect T cells in the patient's blood that responded in vitro to MART-1 or MAGE-3. These T cells were undetectable before the infusion of NY-ESO-1–specific CD4+ T cells but became detectable at a time that coincided with regression of the tumor and persisted for at least 3 months. We found no evidence of T cells that could respond to glycoprotein 100, which was poorly expressed by the tumor (Fig. 3).

DISCUSSION

We describe an in vitro method for the development of CD4+ T-cell clones with specificity for a tumor antigen and the use of one such clone for the treatment of a patient with metastatic melanoma. Development of the method has been difficult, in part because of the paucity of well-defined immunogenic HLA class II–restricted epitopes of tumor antigens and also because of the difficulty in isolating and expanding CD4+ T-cell clones to obtain numbers sufficient for clinical use. The HLA-DP4–restricted peptide epitope of the melanoma-associated cancer–testis antigen NY-ESO-1 is one of the most immunogenic tumor antigens[10,14] and can elicit responses by CD4+ T cells from patients with cancer who have been immunized with NY-ESO-1.[15,16]

Using the NY-ESO-1 peptide, we succeeded in generating NY-ESO-1–specific CD4+ T cells from the blood of an unimmunized patient with meta-static melanoma. We were able to expand these cells to several billion and use them to treat the patient. No exogenous cytokines were administered because these CD4+ T cells, when stimulated in vitro by the NY-ESO-1 antigen, produce auto-crine-active interleukin-2. After infusion of the cells, influenza-like symptoms and lymphopenia, signs of a cytokine release syndrome, developed. In contrast to transferred CD8+ T cells, which survive only briefly (<20 days) in vivo in the absence of exogenous cytokine,[1,3] the CD4+ T-cell clones that we administered without any cytokine treatment were detectable in the patient's blood for at least 3 months. Therefore, the possibility that in vitro–derived and clonally expanded T cells were functionally exhausted[17] was not substantiated by our results.

Our studies and those performed by others have shown that immunotherapy with antigen-specific T cells can eliminate tumor cells that express the corresponding antigen, but it can also allow the outgrowth of antigen-loss tumor variants.[1,3] The target antigen in our case, NY-ESO-1, was not uniformly expressed by the cells of the patient's melanoma, yet the tumor regressed completely. We postulate that the complete regression of the tumor can be attributed in part to responses by the patient's immune system to other antigens displayed by the tumor. This additional response could have come about through antigens in apoptotic bodies that were released from killed tumor cells. When collected and processed by antigen-presenting cells, these antigens probably activated a variety of T cells, thereby broadening the immune response, a process referred to as antigen spreading.[18,19] The delay of days to weeks in the development of these additional responses is consistent with antigen spreading (Fig. 3).

The fact that T cells against glycoprotein 100, an antigen that was not expressed by the melanoma, were undetectable indicates the selectivity of the antitumor immune responses. It is also possible that the reduction in the tumor burden relieved immunosuppression in the

tumor microenvironment caused by inhibitory cytokines and regulatory T cells, thereby contributing to the broadened T-cell response. IgG antibodies against NY-ESO-1 were detectable in plasma samples from the patient before infusion of the T-cell clone and remained unchanged in titer after the infusion. The presence of antibodies against NY-ESO-1 is associated with an increased likelihood that NY-ESO-1–specific CD4+ and CD8+ T-cell responses will be generated ex vivo.[14,20] Antibodies against MAGE-3 and MART-1 were undetectable before the T-cell infusion and afterward for up to 87 days (Fig. 1 in the Supplementary Appendix). The lack of an increase in the titers of antibodies against melanoma antigens despite the appearance of CD4+ T cells against these antigens suggests that the production of such antibodies is independent of T cells.

In summary, we showed that the infusion of a clonal population of CD4+ T cells with specificity for a single tumor-associated antigen caused complete regression of a tumor. During regression of the tumor, this clone appears to have induced the patient's own T cells to respond to other antigens of his tumor. These findings support further clinical studies of antigen-specific CD4+ T cells in the treatment of malignant disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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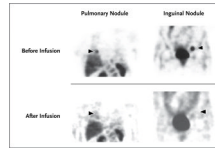
Figure 1. PET Scans Obtained before T-Cell Infusion and 2 Months after Infusion
Preinfusion positron-emission tomography (PET) reveals hypermetabolic lesions in the right lung and the left inguinal–iliac node that are consistent with metastases (arrowheads); the lesions colocalized to tumor nodules on computed tomography (CT). After infusion with NY-ESO-1–specific CD4+ T cells, PET scans show that the tumor nodules have regressed, and no evidence of disease can be detected. Uptake in liver, spleen, and bladder represents normal background signal and did not colocalize to any lesions on CT scans.
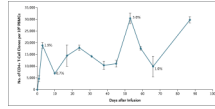
Figure 2. Persistence of Transferred NY-ESO-1–Specific CD4+ T-Cell Clones In Vivo
Quantitative polymerase-chain-reaction (PCR) assay with the use of clone-specific primers
flanking the TCR–CDR3 region was used to determine the frequency of infused CD4+ T-
cell clones among peripheral-blood mononuclear cells (PBMCs) in samples collected before
infusion and at intervals after infusion until day 90. Results are represented as the number of
T-cell clones per 1 million PBMCs and as a percentage of the total number of PBMCs at
peak points and low points. As expected, the NY-ESO-1–specific CD4+ T-cell clone is
undetectable in preinfusion PBMCs (sensitivity of detection, 1:100,000 cells). After
infusion, the frequency of the infused CD4+ T-cell clone in vivo increased to almost 2% of
all PBMCs and fluctuated between 0.7 and 3.0% during the next 12 weeks. The I bars
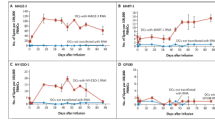denote standard errors.

Figure 3. Augmentation of T-Cell Responses to Melanoma-Associated Tumor Antigens
Antigen-specific T-cell responses to melanoma antigen recognized by T cells (MART-1), melanoma antigen 3 (MAGE-3), NY-ESO-1, and glycoprotein 100 (GP100) were evaluated in samples of peripheral-blood mononuclear cells (PBMCs) collected before infusion and at time points up to 12 weeks after infusion. Autologous dendritic cells (DCs) that were transfected with RNA-encoding tumor antigen were used to stimulate PBMCs in enzyme-linked immunospot assays. The frequency of the antigen-specific response is presented as the number of reactive spots per 100,000 PBMCs (squares). Parallel wells with DCs transfected with control RNA were used at each time point (diamonds). Each data point is an average of the values from triplicate wells. After infusion, marked elevations in antigen-specific T-cell responses were observed for MAGE-3 (Panel A), MART-1 (Panel B), and NY-ESO-1 (Panel C) but not for GP100 (Panel D). The I bars denote standard errors.